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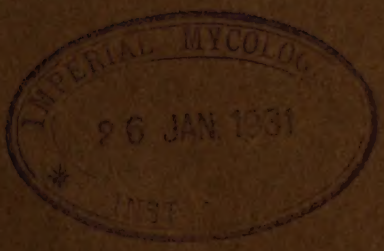


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# STUDIES ON THE MOSAIC DISEASE OF THE BEAN (PHASEOLUS VULGARIS L.)

T. G. FAJARDO[1]

## INTRODUCTION

Within the last decade the cultivation of both field and snap beans, *Phaseolus vulgaris* L., has increased markedly in certain sections of the United States. This has been associated especially with the increase of the bean-canning industry in the North and of the winter culture of beans in the South. At the same time there has been a concentration of the bean-seed-growing industry in the Northwest. Concomitant with these changes has come increasing loss from certain seed-borne diseases. Among these, the virus disease, "mosaic," ranks as most important on certain varieties. Although this has been recognized as serious for some time, but little progress has been made recently in its critical study, partly because of the difficulty in studying this mosaic experimentally.

The investigations here reported are the result of studies conducted at Madison, Wisconsin, dealing principally with certain phases of artificial and natural transmission of the disease, including transmission through the seed.

## HISTORY, GEOGRAPHIC DISTRIBUTION, AND ECONOMIC IMPORTANCE

Bean mosaic was first reported in Russia by Iwanowski (10), in 1899, on a variety of *Phaseolus vulgaris*. Clinton (3), in 1908, described a questionable "infectious chlorosis" of string beans in Connecticut, which "in general appearance resembled very closely the chlorosis (mosaic or calico) troubles of tobacco, tomatoes and muskmelons." Spragg and Down (21) state that the disease was serious on the commercial navy bean in Michigan in 1908. In 1914 the disease was reported as occurring locally and causing slight damage in New York, but in 1916 and 1917 it became more general and severe (17). Barss (2) noted the disease in Oregon in 1917 and found that in certain fields as high as 50 per cent of the plants were affected.

[1] The writer is indebted to Professor L. R. Jones, at whose suggestion this investigation was undertaken, for advice and encouragement throughout the investigation; to Dr. James Johnson for helpful advice; and to Professor H. P. Barss, Dr. S. M. Zeller, and Dr. I. A. Hoggan for aid in the preparation of the manuscript.

Since the appearance of bean mosaic in Connecticut, Michigan, New York, and Oregon, the disease has been reported in various other States and is now apparently coextensive with bean culture in the United States (18) and perhaps in Canada (4, 8, 23). The disease has caused serious losses in the Eastern States in the past, but with the development of "Robust," a chance selection from Michigan, and other resistant varieties of dry shell beans, conditions have improved in this region. In the Western States, however, especially in Montana, Idaho, and other States where commercial seed beans are grown, but where Robust is not, the disease has become the most serious malady with certain important varieties. According to the Bureau of Plant Industry (Plant Disease Survey for 1925 to 1927), bean mosaic was ranked second only to bacterial blight in causing reduction in total yield for the bean crop of the United States (18).

While bean mosaic and the loss caused by it are coextensive with bean culture in America, only meager reports of the disease in other countries have been found in literature. Jørstad (12) reported its occurrence in Sweden in 1920, Ogilvie (16) in Bermuda in 1923, and Porter (19) in Eastern China in 1926. Quanjer, in a letter to the writer in 1927, states that he had observed it often in Holland and also in England. Since the disease is carried through the seed, it is probable that it occurs wherever beans are grown. Presumably the spread from one locality to another is chiefly through commercial shipments of bean seed.

## SYMPTOMS

*General symptoms and effects of bean mosaic.* The symptoms displayed by bean plants affected with mosaic are variable and are influenced by various environmental conditions. In general, the leaves show varying degrees of mottling or chlorosis, blistering, and downward cupping of the lamina (Fig. 1, A, B, and E). Under certain environmental conditions, much-reduced leaves, with sinuous or filiform leaflets, are produced; in some cases only the terminal leaflet develops (Fig. 1, C, D). These latter symptoms, however, have not been observed in the field.

Plants affected with mosaic are rarely killed but continue to vegetate to the end of the season. They may become stunted, dwarfed, and bushy, or may be only slightly affected in growth, especially when the plant becomes infected at a later stage of development. In general, they shed their flowers more freely than do healthy plants and the setting of pods is delayed, the pods not all being set at the same time (Fig. 2). Under these conditions, the plants may be induced to more active budding and the vines appear "green" or take longer to "dry off" than the vines of healthy plants. This is especially noticeable towards the end of the season, when uniform

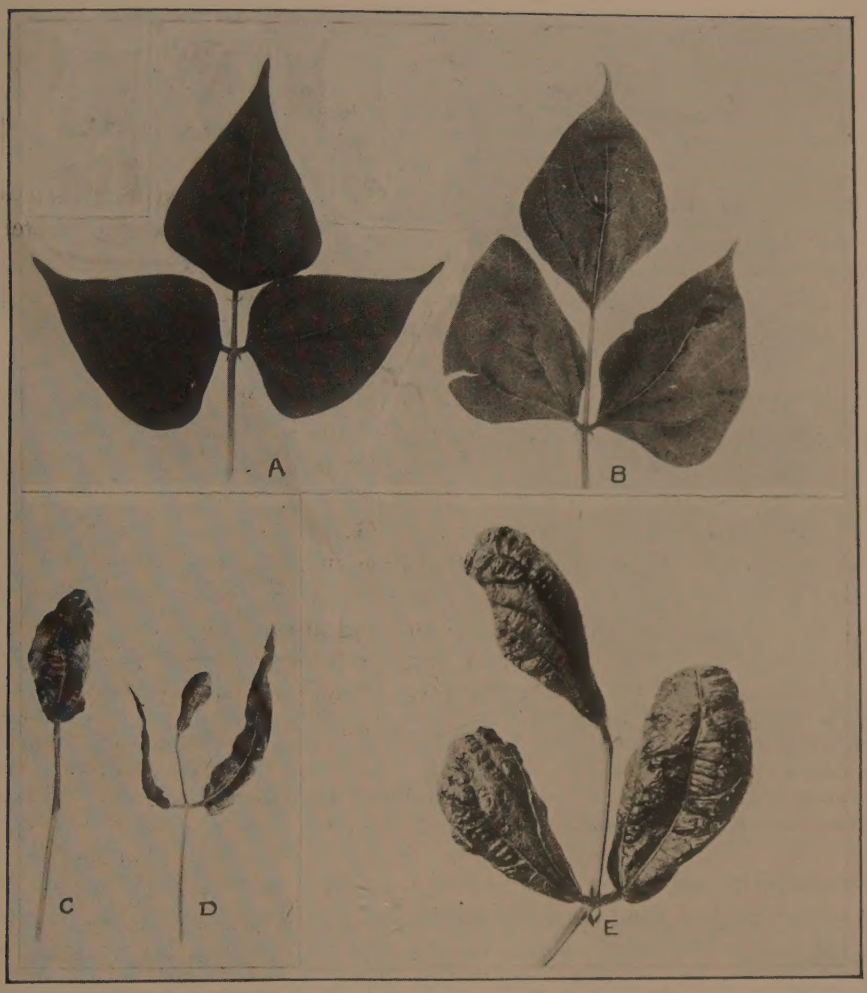


FIG. 1. Variation in leaf symptoms exhibited by different susceptible varieties of beans affected with mosaic.

Top row, Early Marrow Pea. A, leaf of healthy plant. B, leaf of mosaic plant showing chlorosis, with a few, small, dark green areas near the veins.

Bottom row, Rogers Stringless Green Pod. C, severe blistering and malformation of leaflet, with suppression of lateral leaflets. D, filiform malformation of leaflets. E, marked blistering and cupping of leaflets.

yellowing or drying of healthy vines occurs. Plants which become infected late in the season, however, may appear green although their pods mature uniformly and at approximately the same time as those of healthy plants.

The loss in yield, due to mosaic, varies from 0 to 100 per cent, depending on the stage of growth of the plant when infection occurs and on the relative

FIG. 2. The amount of injury caused by mosaic is much greater when infection occurs early. A, plant which developed primary symptoms (indicated by arrow) on August 20 and on which the yield was not reduced. B, plant which developed primary symptoms (indicated by arrow) on July 31, when in the fourth compound-leaf stage, showing delay in setting of pods and corresponding reduction in yield. (Refugee 1000–1 variety, photographed August 25.)

susceptibility and season of the variety. Plants infected early and developing severe leaf symptoms oftentimes fail to produce any pods. Plants infected at a later stage, before or just after blossoming, may be reduced in yield from 30 to 50 per cent, while plants infected after the pods are set are slightly or not at all reduced in yield (Figs. 2 and 3).

*Symptoms on plants infected during the current season.* Healthy plants which become infected during the current season through the agency of insects or through artificial inoculation show the first evident symptoms of mosaic on the young expanding leaves 8 to 15 days after inoculation. The youngest leaves which are expanding at the time of inoculation become crinkled, chlorotic, or stiff, with a much shortened petiole. The next youngest leaves are at first thickened, more or less chlorotic, and laterally arched, but later become completely chlorotic with petioles of normal length (Fig. 4, B). Under favorable greenhouse temperatures, these symptoms may be much more pronounced and a clearing of the veins may

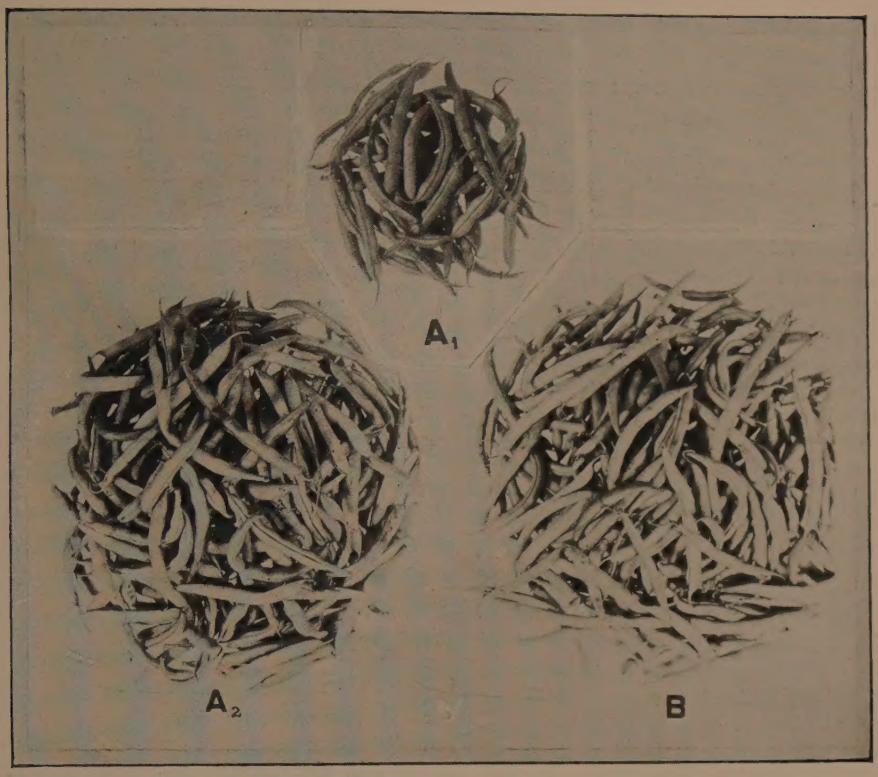


FIG. 3. Comparative yields of healthy and mosaic plants (Refugee 1000–1). $A_1$, yield from 5 plants infected at an early stage of growth. $A_2$, yield from 5 plants developing first symptoms of mosaic when in full blossom or later. B, yield from 5 healthy plants (control).

be observed (Fig. 5). Under certain conditions, however, plants infected during the current season may not develop typical symptoms. Such plants are either growing very slowly or have already reached full vegetative growth before they become infected, or they bcome infected late in the season when the weather is cool and moist. These latter plants may show only a crinkling or chlorosis of the last compound leaves; but, when conditions become more favorable for growth and the air temperature becomes higher, new leaves may be produced which show typical mosaic symptoms.

*Symptoms on plants originating from infected seed.* As will be discussed later, bean mosaic is often transmitted through the seed, although not all the seeds from an infected plant show mosaic infection. Seedlings originating from infected seed vary in the time of appearance and severity of leaf symptoms. Table 1 illustrates the results obtained with mosaic parent plants in relation to the time of appearance of symptoms in their

TABLE 1.—*Relation of time of infection of the parent plant to the percentage of seed infected. (Note the larger percentages result from early inoculation)*

| Stage of growth of parent plant when infected | Number of experimental plants | Total number of seeds secured and tested | Percentage of seedlings from these developing mosaic symptoms | |
|---|---|---|---|---|
| | | | After 20 days[a] | After 30 days[a] |
| 2nd to 4th compound leaf... | 10 | 163 | 44 | 57 |
| 5th to 7th compound leaf..... | 5 | 171 | 25 | 36 |

[a] Beginning from the date on which the seeds were planted.

FIG. 4. A, seedling plant 20 days old, grown from infected seed and showing typical mosaic symptoms on both simple and compound leaves. B, plant artificially inoculated in the second compound-leaf stage, showing primary symptoms on the second and third compound leaves (indicated by arrows) and typical symptoms on subsequent leaves. (Roger's Stringless Green Pod.)

progeny. Our observations indicate that some seedlings develop typical mottling, blistering, downward cupping, or chlorosis on the first and succeeding compound leaves, while the two simple leaves are chlorotic, mottled or contorted, or else normal in appearance (Fig. 4, A). Sometimes only one of the simple leaves shows any symptoms. On other plants, however, even the first compound leaves do not develop typical symptoms but show a stiffening, crinkling, or chlorosis of the lamina, while the petiole becomes shortened and thickened and subtends the lamina in a more or less upright

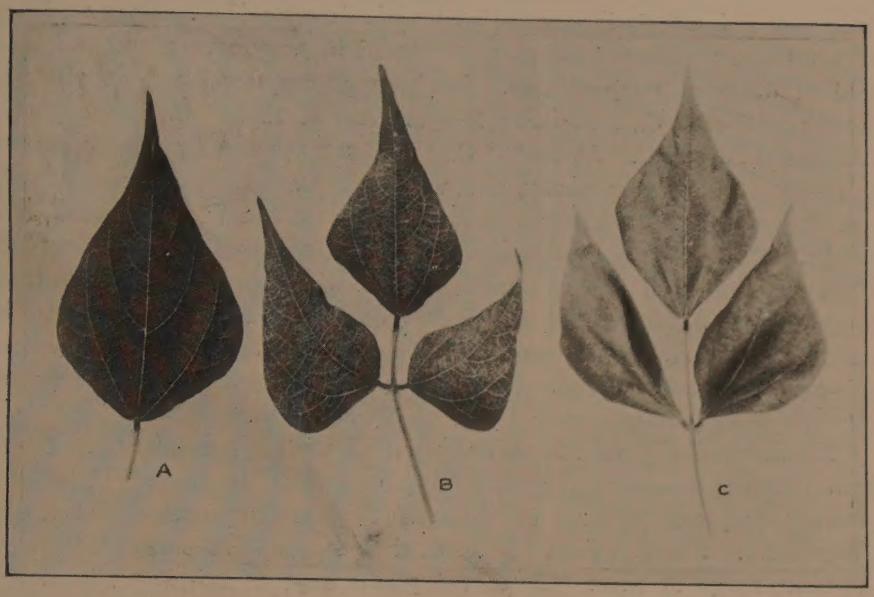


FIG. 5. Primary mosaic symptoms resulting from artificial inoculation in the greenhouse (Roger's Stringless Green Pod). A, leaflet of healthy plant. B, leaf of mosaic plant showing marked clearing of the veins. C, leaf of mosaic plant showing general chlorosis.

position. The second compound leaves are chlorotic, and typical symptoms develop on subsequent leaves. Thus far, it has not been possible to find any explanation of the fact that different plants of the same variety, grown from the same lot of infected seed and under identical conditions, should vary thus in the time of appearance of symptoms.

The severity of leaf symptoms on plants grown from infected seed was found to vary widely according to the variety. Generally speaking, the early, apparently resistant, varieties do not show such well-marked mottling, blistering, or cupping as is observed on the late susceptible varieties. The early, apparently resistant varieties, such as Bountiful, Davis White Wax, Pencil Pod Wax, Wardwell's Wax, and Giant Stringless Green Pod, show only a slight mottling or chlorosis of the leaves. Sometimes the symptoms on the compound leaves are obscured in the greenhouse, even though the simple leaves show marked contortions. Under greenhouse conditions these varieties blossom very early, which may explain the failure in some cases of the appearance of mosaic symptoms. Our experience indicates also that both these varieties and the late-maturing types, unless inoculated early, may fail to develop mosaic symptoms.

*Influence of air temperature on the expression of mosaic symptoms.* Air temperature affects the general symptoms of bean mosaic in much the

same way as it affects those of other mosaic diseases (11). Bean seedlings with marked mosaic symptoms were placed in greenhouses and chambers held at different temperatures. Good symptoms developed on the new growth at 20–28° C., a masking of symptoms at 12–18° C., and a partial masking at 28–32° C. At 20–28° C., the plants apparently grew normally, at 12–18° C. they were stocky, and, at 28–32° C., they grew more rapidly and were spindly. Field observations during 1926 and 1927 confirm these results. Marked symptoms were noted in the field from about the last half of June to the first two weeks of August, when the mean temperatures ranged between 20 and 29° C. Before and after this period, when the mean temperatures lay between 10 and 18° C., the symptoms were indistinct or were completely masked. The masking of symptoms was more noticeable late in the summer when cooler weather ensued.

The incubation period is also affected by the air temperature. Under good growing conditions, inoculated plants kept at 20–28° C. showed primary symptoms within 8 to 14 days. Typical symptoms appeared 3 to 6 days later. Plants kept at 28–32° C. showed symptoms in approximately the same range of time. Inoculated plants kept at 12–18° C. showed a lengthening of 4 to 7 days in the incubation period and failure to develop typical symptoms even after 25 days. When transferred to a temperature of 22–26° C., however, typical symptoms appeared in 10 days. In these experiments from 60 to 100 per cent infection was obtained with plants kept at 12–18° C. and at 20–28° C. Much lower percentages of infection were obtained with plants kept at 28–32° C. The low percentages of infection obtained with inoculated plants at the higher temperatures may have been due to the sensitiveness of the virus to drying or to the rapid drying of the mutilated leaves at these temperatures.

Judging from the above results, the favorable temperatures for the expression of mosaic symptoms are 20–28° C., with an optimum between 22 and 26° C.; partial masking occurs at 28–32° C., or above, and total masking at 12–18° C. At favorable temperatures (20–28° C.) typical mosaic symptoms develop within 15 to 20 days, a much shorter period than that given by Stewart and Reddick (22), which was 4 weeks.

### IS THERE MORE THAN ONE KIND OF BEAN MOSAIC?

Since great variations in mosaic symptoms have been found among the different varieties of beans and since field-infected plants under certain conditions fail to develop typical mosaic symptoms during the season, one might be led to suspect the existence of more than one kind of mosaic on bean. The writer, however, is at present of the opinion that there is only one common type of mosaic of the bean. It has not been possible to make

extensive intervarietal cross inoculations and studies of the properties of the virus derived from different sources. This conclusion, however, is based, first, on the results of a study of symptom development in a series of experiments with young susceptible plants, where inoculations were made with infective materials taken from plants and varieties showing diverse symptoms in the field, and, second, on observations of the diverse effects of the disease on different plants raised from diseased seeds taken from the same infected parent plant. The variations observed in the expression of symptoms seem to be due to differences in host reaction as influenced by a variety of internal and external factors.

## DISTRIBUTION OF THE VIRUS IN INFECTED PLANTS

The virus of bean mosaic is perhaps present in the whole system of mosaic-infected plants, but its presence is most easily demonstrable in those plant parts which exhibit mosaic symptoms. Artificial inoculations with juice from the leaves, stems, or green pods of mosaic-infected plants readily produce infection in young, healthy bean plants. No infection has thus far been obtained from the juice of roots of infected plants. Several attempts to inoculate young seedlings with water extracts from dried seed coats of mosaic-infected seed carrying 60 to 75 per cent seed infection gave negative results. Surface sterilization of infected bean seeds had no appreciable effect, and this held true whether or not the seed coats were removed before sterilization. This indicates that virus is, perhaps, not present in the seed coat but is carried in the embryo of the seed. No attempts have been made to inoculate with the juice from fresh seed coats. It is possible that the virus may be present in them, but, if so, it probably loses its virulence and becomes inactive with the drying of the seed coat.

## TRANSMISSION OF BEAN MOSAIC

*Transmission through the soil.* In several series of experiments no transmission of mosaic was obtained through the soil. In these experiments seeds of susceptible varieties of beans were planted in soil mixed with fresh, dried, or decomposed mosaic tissues, or the seeds were allowed to sprout through a layer of fresh, mosaic-infected bean leaves. These results indicate that the virus of bean mosaic probably does not overwinter in dead, diseased vines and is perhaps never transmitted through the soil. As will be discussed later, the bean-mosaic virus remains viable an exceedingly short time and will not resist drying.

*Transmission through contact with diseased and healthy roots.* No transmission has yet been obtained in several series of experiments conducted in an attempt to infect healthy plants through contact with the

roots of mosaic plants. In these experiments seeds of healthy and mosaic plants were planted in the same pot. In no case could transmission be ascribed to root contacts. The healthy seedlings remained healthy, although the seeds from the mosaic plants gave a high percentage of infected seedlings.

*Transmission through contact with the aerial parts of diseased and healthy plants.* Leaves of healthy and mosaic plants, allowed to come in contact with each other and casually rubbed together under controlled conditions, failed to cause infection of the healthy plants. Alternate picking of green pods from mosaic and healthy plants grown under cages likewise gave no infection. The results of these trials indicate that bean mosaic is not readily transmissible through cultural practices, which must therefore be considered of little or no economic importance in the transmission of bean mosaic in the field.

### TRANSMISSION BY ARTIFICIAL MEANS

*Seed inoculation.* No infection was obtained when dry or germinating seeds from healthy plants were soaked in juice expressed from diseased plants for different lengths of time. Pricking the cotyledons of germinating seeds before soaking in infective juice for 12 hours failed to induce infection. The juice was proved still to be infectious, after 12 hours, by inoculation to healthy plants.

*Root inoculation.* In two series of experiments, successful infection was, however, obtained when the cut roots of seedlings were immersed in infective expressed juice for various lengths of time. From 20 to 40 per cent infection was obtained on seedlings in the simple-leaf stage after such soaking for 1 to 1½ hours and on plants with the simple leaves half expanded after soaking 3 to 5 hours. No infection was secured on seedlings in the cotyledonary stage after soaking for 12 hours. Older plants subjected to this method died after replanting, as did some of the plants in the simple-leaf stage. These results suggest that infection resulted thus through cut roots only when sufficient transportation occurred through aerial parts to induce fairly active absorption and distribution of the virus.

*Leaf inoculation.* To date, bean mosaic compared with the well-known tobacco and cucumber mosaics, has been considered difficult to transmit artificially. Although transmission was secured by the earlier workers, the percentage of infection obtained was low and the results inconsistent (20, 7), while others failed to obtain transmission (6, 3). Clinton (3), failing to transmit the disease, regarded it as a "questionable chlorosis." Reddick and Stewart (20) state that they failed "in producing infection with expressed juice of diseased plants either with sufficient regularity or in

sufficient quantity to feel sure that the occasional plants showing the disease had not become infected in some other way.'' Archibald (1) states, however, that he obtained 100 per cent infection with hypodermic injections and by pressing the leaves between the fingers moistened with extracts from mosaic plants.

Using the methods of artificial inoculation known to transmit tobacco or cucumber mosaic and also repeating some of the methods used by the earlier workers with bean mosaic, the writer also has found the disease difficult to transmit. A method of leaf-mutilation inoculation has been developed, however, which has given from 80 to 100 per cent of infection in the majority of cases. This method consists in rubbing the simple leaves on the upper surface along the midrib with a small piece of cheesecloth dipped in infective, expressed juice to which a small quantity of sand has been added. Satisfactory results also were obtained by rubbing the leaves with mosaic tissues which were ground up and mixed with sand and rolled into small balls. These same methods applied to other small areas of the simple leaves have given satisfactory infection, but this has not been so commonly practiced as the rubbing of the midrib. The addition of sand in the juice, which is readily taken up with the cheesecloth, greatly aids uniform abrasion of the rather tough and firm leaves of the bean plant and avoids unnecessary tearing and crushing. By these methods, infection of susceptible varieties has been secured at any stage from the simple-leaf to the flowering stages.

*Transmission by insects.* The spread of certain virus diseases has been largely ascribed to insect agencies, and bean mosaic has been assumed by earlier workers to be transmitted by insects also. Our early field trials showed that if susceptible bean plants were so caged as to exclude insects they remained healthy through the season, while exposed controls developed mosaic symptoms before the end of the season. Later experiments, however, have proved more conclusively that certain aphids readily transmit bean mosaic, while all other various insects tested have failed to give infection.

*Transmission by aphids.* Hawley (9) states that the work of Matheson and Reddick indicated the transmission of bean mosaic by an undetermined species of plant louse in the greenhouse. Nelson (15) showed that *Macrosiphum solanifolii* Ashm., the pink and green potato aphid, transmitted bean mosaic. The results of a number of cage experiments with mosaic-reared *Aphis rumicis* L., the black bean aphid, *Myzus persicae* Sulzer, the peach aphid, and *Macrosiphum solanifolii*[2] have shown that each of these

[2] The writer wishes to thank Dr. A. A. Granovsky of the Department of Economic Entomology, University of Wisconsin, for kindly identifying the aphid species.

species readily transmits the bean-mosaic virus to healthy bean plants, confirming Nelson's results with the last-named aphid. Eighty to 100 per cent infection was secured when 15 or more infective aphids were transferred to each individual experimental plant. Lower percentages of infection were obtained when fewer aphids were used. In one series of experiments, infection was obtained with a single viruliferous individual of *A. rumicis* and like results with *Myzus persicae*. The incubation period remained within the usual range whether many or few aphids were transferred to each plant. Figure 6 is a general view of the plot where insect transmission experiments were conducted.

FIG. 6. General view of bean plot showing cages under which insect-transmission experiments were conducted.

*Transmission by other insects.* With one exception tests with various other insects found feeding on bean plants in the field gave negative results. Several trials with the bean leaf hopper, *Empoasca fabae* Le B., reared on mosaic bean plants, have given negative results. No infection was obtained either from *Diabrotica duodecimpunctata* Oliv., the twelve-spotted cucumber beetle, *D. vittata* Fabr., the striped cucumber beetle, *Lygus pratensis* L., tarnished plant bug, *Tetranychus telarius*, red spider, *Haliothrips fasciatus*, thrips, or *Aleyrodes vaporarium* Westw., white fly. Positive infection was obtained, however, with a species of mealybug in the greenhouse.

TABLE 2.—*Summary of results of 1926 trials as to the amount of field transmission attributed to insects in bean plots at Madison, Wisconsin. (Plantings made from June 3–5)*

| Variety | Plot I | | | Plot II | | |
|---|---|---|---|---|---|---|
| | Total No. of plants | Percentage of seed-borne mosaic | Percentage of mosaic plants on Sept. 15 | Total No. of plants | Percentage of seed-borne mosaic | Percentage of mosaic plants on Sept. 15 |
| *Early Varieties* | | | | | | |
| Giant Stringless Green Pod | 132 | 2.2 | 33.3 | 74 | 1.3 | 98.7 |
| Bountiful | 82 | 1.0 | 56.3 | 86 | 0.0 | 100.0 |
| Davis White Wax | 75 | 0.0 | 8.0 | 36 | 0.0 | 100.0 |
| Improved Golden Wax | 58 | 0.0 | 29.1 | 15 | 0.0 | 73.0 |
| Refugee Wax | 55 | 3.6 | 47.3 | 74 | 0.0 | 100.0 |
| Dwarf Horticultural | 105 | 4.7 | 35.0 | 64 | 0.0 | 100.0 |
| Extra Early Refugee | 108 | 0.0 | 38.9 | 67 | 0.0 | 100.0 |
| Wardwell Wax | 30 | 0.0 | 26.7 | 25 | 0.0 | 100.0 |
| Full Measure | 32 | 0.0 | 34.4 | 17 | 0.0 | 100.0 |
| Grennel Wax | 47 | 0.0 | 21.0 | 15 | 0.0 | 46.0 |
| *Late Varieties* | | | | | | |
| Refugee 1000–1 | 132 | 2.2 | 33.2 | 74 | 1.3 | 98.7 |
| Roger's Stringless Green Pod | 116 | 15.5 | 84.5 | 63 | 3.2 | 96.8 |
| Hodson Wax | 70 | 20.0 | 80.0 | 74 | 5.4 | 94.6 |
| Early Marrow Pea | 62 | 0.0 | 43.5 | 70 | 1.4 | 98.6 |
| Large Marrow Pea | 95 | 1.0 | 35.8 | 72 | 0.0 | 100.0 |
| Robust | 75 | 0.0 | 0.0 | 112 | 0.0 | 0.0 |

### FACTORS INFLUENCING MOSAIC TRANSMISSION IN THE FIELD

The rate of spread and final percentage of mosaic infection in our experimental bean plots have been observed to vary from season to season. The determining factors appear to be (1) the percentage of mosaic-infected seed, and (2) the prevalence of certain insects. Climatic conditions may influence either the development of insects or the expression of mosaic.

In 1926 samples of seed of representative commercial varieties of beans, which carried low percentages of infection, were planted in early June in parallel rows in each of three different plots. The percentage of plants showing infection in two of these, plots I–II, at the end of the season, is given in table 2. The same variety showed differences in the percentage of infected plants in the different plots when final notes were made. The rows planted in plot II, where aphids appeared early in July and were more abundant during the season, gave a higher percentage of infection than those planted in the other two plots. In the latter plots aphids appeared late and were not abundant throughout the season. The rate of spread of mosaic was here much slower than in plot II. These results were confirmed by those obtained in 1927. In 1927 seeds from selected healthy plants were planted in rows alternately with mosaic seeds. Plot II showed 100 per cent infection by the end of July, while only a few cases of field infection were observed at that date in the other two plots. These other plots, however, also showed 100 per cent infection at the end of the season. The high percentages of field infection observed as compared with the previous year were due to the higher percentages of infected seed planted in the plots. The prevalence of aphids was similar to that of the preceding year.

### INFLUENCE OF ROGUING ON AMOUNT OF FIELD INSPECTION

Roguing was found to have some influence on the prevalence of mosaic when the amount of seed infection was low and the roguing began with the earliest seedling symptoms before the appearance of aphids. Thus in one trial where, in the nonrogued controls, the infection of the more susceptible varieties reached 30 per cent, the corresponding rogued plots showed not more than 10 to 20 per cent even on the late susceptible varieties. Although this represented a reduction to almost one-half, it cannot be considered a practical measure under ordinary conditions.

Our experiments have shown that the amount of field infection attributed to insects and the resulting mosaic injury may vary widely according to date of planting. This is illustrated in table 3, which shows the results from each of three plantings, early June, late July, and early August. It will be noted that the two earlier plantings gave high infections on all

TABLE 3.—*Relation of date of planting to the amount of mosaic infection at the end of the season (1926 trials, Plot II). (Note the very small amount of mosaic in the August planting when there were few insects)*

| Variety | Planted June 10 | | Planted July 23 | | Planted August 5 | |
|---|---|---|---|---|---|---|
| | Total No. of plants | Percentage mosaic on Sept. 10 | Total No. of plants | Percentage mosaic on Sept. 10 | Total No. of plants | Percentage mosaic on Sept. 10 |
| *Early Varieties* | | | | | | |
| Refugee Wax | 19 | 100.0 | 55 | 100.0 | 21 | 0.0 |
| Giant Stringless Green Pod | 24 | 100.0 | 20 | 100.0 | 15 | 13.3 |
| Bountfiul | 40 | 100.0 | 46 | 100.0 | 20 | 10.0 |
| Davis White Wax | 20 | 100.0 | 17 | 100.0 | 20 | 5.0 |
| Pencil Pod Wax | 30 | 66.7 | 22 | 75.0 | 25 | 8.0 |
| Improved Golden Wax | 15 | 73.3 | 25 | 100.0 | 15 | 0.0 |
| Dwarf Horticultural | 35 | 100.0 | 29 | 100.0 | 26 | 0.0 |
| Extra Early Refugee | 28 | 100.0 | 39 | 100.0 | 25 | 0.0 |
| Full Measure | 15 | 100.0 | 18 | 100.0 | 25 | 0.0 |
| Sure Crop | 15 | 100.0 | 39 | 100.0 | 15 | 0.0 |
| Refugee Wax (caged control) | 10 | 0.0 | 15 | 0.0 | 25 | 0.0 |
| *Late Varieties* | | | | | | |
| Refugee 100–1 | 29 | 100.0 | 25 | 100.0 | 25 | 4.1 |
| Roger's Stringless Green Pod | 25 | 100.0 | 45 | 100.0 | 15 | 20.0 |
| Robust (resistant) | 45 | 0.0 | 42 | 0.0 | 25 | 0.0 |
| Refugee 1000–1 (caged control) | 10 | 0.0 | 30 | 0.0 | 25 | 0.0 |

susceptible varieties except where caged to exclude insects. In sharp contrast those planted in August were relatively free from mosaic. In another like but larger plot, in 1926, the results were similar, with mosaic infection of 60 to 100 per cent on the two earlier plantings (June and July). Alongside these were plantings on two later dates of which that made August 1 showed small infection viz, 0 to 20 per cent, while that planted August 15 escaped entirely. The explanation seems to lie in the fact that the August plantings broke through the ground at a time when the insects had almost entirely disappeared, and hence they escaped infection.

Like results were obtained in 1927, when seeds from selected healthy plants of ten susceptible varieties were planted on seven successive dates in duplicate plots numbered I and II. The planting dates were: May 20–25, June 6, June 10, July 1, July 15, August 1, and August 15. The results are summarized in table 4. These findings indicate that when the seed is

TABLE 4.—*Further data on the relation of the date of planting to the amount of mosaic infection at the end of the season (1927 trials on ten susceptible varieties). (Note the escape from infection of those planted in May as well as those planted in August)*

| Date of planting | Percentage of mosaic plants at end of season | |
|---|---|---|
| | Plot I | Plot III |
| May 20–25 | 0–20 | 0– 40 |
| June 6–July 15 | 50–80 | 80–100 |
| August 1–August 15 | 15–25 | 15– 60 |

planted early, the plants have a greater chance of escaping infection and suffer less damage from mosaic than when the seed is planted much later. Plants resulting from the May planting were vegetatively mature and in full blossom or podding stages when subjected to infection through insects. Plants subjected to infection at late stages in their growth, as was mentioned earlier, are less seriously affected by mosaic or may even fail to produce typical symptoms of the disease. The late-maturing varieties, however, owing to their longer growing and flowering periods, are hardly able to escape infection in the field and subsequent damage from mosaic, even when planted as early as May 20–25. These varieties, even though close to the flowering stage, are still growing actively when insects appear in July. The early-maturing varieties, on the other hand, are already in the blossoming or podding stages at this date. The amount of injury suffered by the early varieties, however, increases with the lateness in planting date.

While the percentage of infection was low on the August 1 and August 15 plantings, planting at these late dates is of no practical value under Madison climatic conditions, since most of the varieties would still be in the flowering or early podding stages in September when frosts may occur. Our results suggest, however, that when early or late commercial beans may be planted early in May, the amount of damage resulting from field infection may be reduced materially.

Such plantings, even though subjected to high insect transmission and showing 100 per cent infection later in the season, are less injured by mosaic, since they will have reached the flowering or podding stage before infection occurs. It is probable that in other regions, also, some advantage may be gained if planting dates can be so regulated as to prevent the period of maximum vegetative activity of the bean from coinciding with the period of maximum aphid activity.

### SEED TRANSMISSION

Transmission of mosaic through seeds of legumes has been reported by Kendrick and Gardner (13) for soy bean, by McClintock (14) for lima bean, and by Dickson (5) for pea, red clover, alsike clover, and sweet pea; but bean mosaic provides, perhaps, the best known example of seed transmission of a virus disease. In their tests Reddick and Stewart (20) found that 50 per cent or more of the seeds from infected parents produced mosaic plants. Archibald (1) and others have likewise found that bean mosaic is transmitted through the seed. In the present investigations, a great variation in the amount of seed transmission was observed in different varieties of commercial beans, and the percentage of seed infection also has been found to vary with the stage of growth when infection of the parent plant occurred, as will be discussed later.

In tests made in 1926 and 1927 as high as 60 per cent of seed infection was found in seed of different late susceptible varieties on the market. Some of the seed lots from the same varieties, however, did not show such high infection. From table 5 it will be seen that, in general, the early varieties show much lower percentages of seed infection. The immune variety, Robust, and a few early varieties, showed no seed infection.

In these studies no evidence has been found that the relative vigor or viability of bean seeds is affected by the presence of the mosaic virus within them. Seed harvested at like stages of maturity appear to germinate equally well whether developed on mosaic or on healthy plants. It has been found that part of the seeds in a single pod may be mosaic-infected and part may be noninfected. In such case both infected and noninfected germinate with equal readiness and vigor. This fact that the presence of

TABLE 5.—*Percentage of seed infection in samples of commercial bean seed*

| Variety | 1926 test | | 1927 test | |
|---|---|---|---|---|
| | Total number of plants | Percentage mosaic | Total number of plants | Percentage mosaic |
| SNAP BEANS | | | | |
| *Early Varieties* | | | | |
| Giant Stringless Green Pod | 215 | 3.5 | 280 | 0.0 |
| Bountiful | 236 | 4.0 | 270 | 0.0 |
| Davis White Wax | 205 | 0.0 | 270 | 1.5 |
| Pencil Pod Wax | 259 | 1.5 | 265 | 2.0 |
| Tennessee Green Pod | 195 | 1.0 | 280 | 4.3 |
| Improved Golden Wax | 140 | 0.0 | 280 | 0.0 |
| Refugee Wax | 300 | 10.0 | 280 | 5.0 |
| Dwarf Horticultural | 180 | 2.8 | 255 | 2.4 |
| Wardwell Wax | 150 | 5.0 | 125 | 3.0 |
| Full Measure | 235 | 0.0 | 270 | 3.0 |
| Golden Wax | 95 | 1.1 | 270 | 1.5 |
| Sure Crop | 125 | 0.0 | 270 | 0.0 |
| Black Valentine | 155 | 0.0 | 275 | 0.0 |
| *Late Varieties* | | | | |
| Roger's Stringless Green Pod | 276 | 15.9 | 945 | 52.1 |
| Refugee 1000–1 | 288 | 13.8 | 270 | 55.5 |
| Hodson Wax | 115 | 19.1 | 255 | 13.4 |
| FIELD BEANS | | | | |
| Early Marrow | 242 | 15.0 | 250 | 30.0 |
| Large Marrow | 175 | 5.0 | — | — |
| White Navy Pea | — | — | 85 | 18.0 |
| Mexican Bean | — | — | 57 | 5.3 |
| Robust | 250 | 0.0 | 704 | 0.0 |

the virus within the seed does not influence germination has material significance as bearing on the seed transmission of the disease.

### RELATION OF THE STAGE OF GROWTH OF THE PARENT PLANTS WHEN INFECTED TO THE AMOUNT OF SEED INFECTION

Our experiments have shown that the amount of seed infection is related to the stage of growth of the parent plant at which infection occurs (Table 1). Plants arising from infected seeds or healthy plants which became infected during early vegetative growth produced more infected seed than did those which became infected at later stages. The appearance of flowers marks the turning point in this respect, since plants infected after the

flowers were set produced almost no infected seed. These facts are illustrated diagrammatically in figure 7, which is based on the results obtained in tests of 1927.

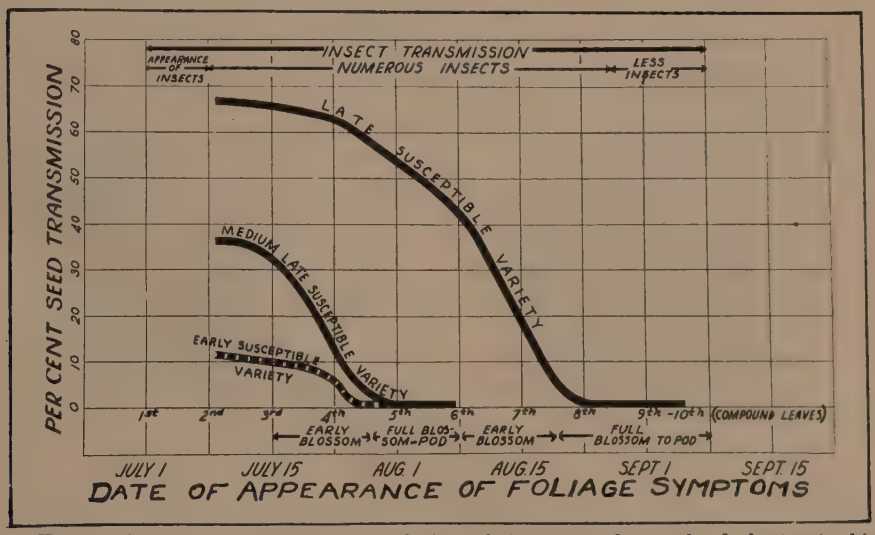


FIG. 7. Diagram illustrating the relation of the stage of growth of plants at which infection occurs to the percentage of mosaic transmission resulting in the seed. The date of appearance of foliage symptoms was used as an indication of the stage at which infection took place. The period of insect activity is indicated at the top of the diagram.

A wide difference was noted between the amount of seed infection occurring in the early and the late varieties. Early varieties infected during the second or third compound-leaf stage showed less seed infection than late varieties infected at the same stage. We believe this is due to the difference in relative stage of growth of the plants at the time of infection. The early varieties blossom and form pods at the third to fifth compound-leaf stage, so that plants infected at the second or third compound-leaf stage will be blossoming or podding when symptoms appear, and most or all of the seeds will escape infection. The late varieties, on the other hand, even if not infected until the fifth or seventh compound-leaf stage, will show symptoms long before the flowers are set; consequently a greater number of seeds will be exposed to infection. These facts may therefore explain why late varieties show relatively more seed transmission of mosaic than do early varieties.

### RELATION OF THE STAGE OF MATURITY OF THE PODS TO THE AMOUNT OF SEED INFECTION

The results of a number of tests showed that, from the same mosaic plant, pods at different stages of maturity varied in the percentage of in-

fected seeds within them. This fact is illustrated diagrammatically in figure 8. The trials were made on the late susceptible Green Refugee

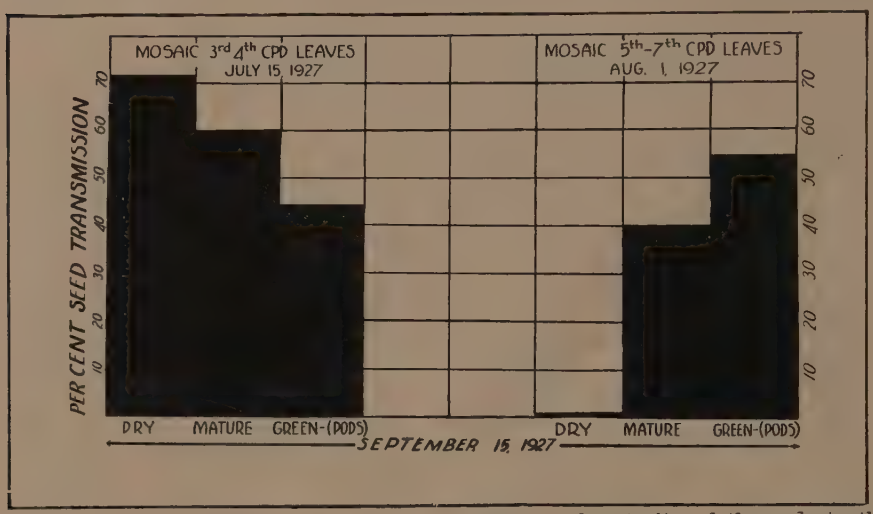


FIG. 8. Diagram illustrating the relation of the stage of maturity of the pods to the amount of seed infection. The pods were picked on September 15, 1927, and the seeds subsequently tested in the greenhouse (Roger's Stringless Green Pod).

variety. When the plants were infected early (third to fourth compound-leaf stage) a high percentage of infection was obtained from the older, dry, ripe pods; whereas, the pods set later, both mature and immature, showed a lower percentage of infected seeds. When the plants were infected later (fifth to seventh compound-leaf stage) there was little or no seed infection in the early-set pods, but the amount of seed infection increased greatly in the pods set later. When infection was further delayed to the full-podding stage, no seed infection was found in any of the pods. This last statement is, however, based on a test with only 10 plants.

No tests have been made with early varieties, but it is believed that these would give similar results, although the range of variation might be less because of the shorter growing and blossoming periods.

The position of the pods on the main stem did not appear to have any direct effect upon the amount of seed infection.

### RELATION OF THE DATE OF PLANTING TO THE AMOUNT OF SEED INFECTION

Planting the seed at different dates influences not only the amount of mosaic occurring on the plants in the field but, also, the amount of seed infection in a given variety. Since, as shown above, the amount of seed infection is directly correlated with the developmental stage of the plants

when infection occurs, this is only to be expected. Our results indicate that beans planted early in May have a greater chance to escape infection through insects, and consequently infection of the seed, than do those planted later, i.e., between June 6 and July 16. Varieties planted in May will have reached the third or fourth compound-leaf stage by the beginning of July when they become liable to insect infection, while those planted later are exposed to infection as soon as they break through the soil. Since it takes about two weeks for symptoms to appear after infection, the plants in the May plantings, infected on or after July 1, will have already reached the blossoming or podding stage when symptoms appear and few or none of their seeds will become infected. Those planted in June or July are exposed to insect infection at much earlier stages and develop mosaic symptoms before the pods are set. Such plantings have, therefore, an increased chance of seed infection.

The most favorable time for planting depends upon the variety and the abundance of insects. In one test, early varieties planted between June 15 and July 15, in a field where insects were scarce, showed little or no seed infection at the end of the season. Where aphids are numerous and infection general, such varieties have increased chances of seed infection as the planting date is delayed. The late varieties, however, on account of their longer growing period, always yield a high percentage of infected seed, even though planted in May or June. Nevertheless, the amount of seed infection is lower than when the planting is done at a later date.

No explanation can yet be offered to account for the reduction in amount of seed infection in plants inoculated at later stages of growth.

## CROSS-INOCULATION STUDIES

No success has been yet obtained in many attempts to transmit the virus of bean mosaic to other leguminous or nonleguminous hosts by various methods of inoculation, nor has any infection resulted when inoculations were made by the usual leaf-mutilation method from mosaic-infected leguminous or non-leguminous hosts to young, healthy bean plants. These results indicate, as suggested earlier, that the mosaic disease of the bean is, perhaps, specific to the bean plant, *Phaseolus vulgaris*.

This idea is consistent with our observations that the initial amount of mosaic development in a given bean field is directly correlated with the percentage of infected seed that is planted. The problem of mosaic control with bean mosaic is, therefore, much simpler than with those virus diseases where there may be various other neighboring host species.

### RELATIVE SUSCEPTIBILITY OF THE BEAN PLANT TO MOSAIC INFECTION AT DIFFERENT STAGES OF GROWTH

Our experiments and observations indicate that susceptible varieties of bean may be readily infected by the virus of bean mosaic at any stage of development except that attempts to infect seedling plants in the cotyledonary or very early simple-leaf stage have thus far given negative results. The variety Robust has not yet been infected at any stage of its development, either artificially or when exposed to high insect transmission in the field. This is in accord with its reputation for immunity from mosaic. Nearly all the other commercial varieties of bean have been tested, however, and all these are readily infected and develop leaf symptoms in different degrees of severity.

### SUGGESTIONS FOR THE CONTROL OF BEAN MOSAIC

The studies here reported were taken up with the object of extending our information, not only on the fundamental aspects of the disease, but also on the problem of control. Our knowledge of the disease is still so incomplete that further studies are necessary for the elucidation of many points relating to the disease and its prevention.

Since bean mosaic, so far as known, overwinters only in the seed and is spread from one locality to another through infected seed in commerce; since the spread in the field is, according to our evidence, accomplished largely if not entirely by aphids, and the extent of field infection is influenced not only by the abundance of these insect carriers but also by the number of plants originating from infected seed; and since the amount of seed transmission is related to the stage of growth when the plants are infected, the following methods of control are suggested as being in accord with the experimental results presented in this paper.

(1) *Production of mosaic-free seed should be accomplished:* (a) By selecting for seed purposes only healthy plants. Selection must be made before the symptoms of mosaic are masked. Plants with masked symptoms can, however, generally be recognized as diseased by examination of the lower leaves, which retain mosaic symptoms; (b) if such healthy plants cannot be obtained, then proceed by selecting plants infected late in the season, preferably those that were not infected until the early- or full-pod stages; (c) by establishing isolated seed plots. These should be planted with seed from carefully selected mosaic-free plants. If this is not available, then use should be made of seeds carrying very low percentages of infection. Such seed plots should be located at some distance from other bean fields and carefully watched with prompt roguing of mosaic plants continued until the blossom stage; (d) if no seed plot is maintained then

the general field from which seed is to come should be similarly rogued until the blossom stage; (e) by adjusting the date of planting, if possible, to bring the varieties to full vegetative growth and to the flowering stage before insects become active. Our trials have shown that, under the conditions of our experimental fields, nearly complete avoidance of seed infection may be attained by early planting of many excellent varieties.

(2) *Seed should be sown which is healthy or contains only a very low percentage of infection.* It appears to be entirely feasible to test lots of bean seed by growing representative samples in index plots, or in the greenhouse, to determine the percentage of infected seed in advance of the time for general field planting.

(3) *Resistant varieties should be used where practicable.* Robust is the best of these, if a white field bean is desired. In order to meet the needs with other types parent plant selections and hybridization should be encouraged.

(4) *Mosaic escape.* Attention should be given to the evidence that either early- or late-planted beans may escape insect infection. It is noteworthy also that the late-maturing varieties suffer most; therefore, attempts should be made to produce varieties maturing early enough to lessen infection, especially that reaching the seed, and which are otherwise satisfactory as to yield and quality.

## SUMMARY

(1) Bean mosaic is coextensive with bean culture in the United States. It is one of the more serious diseases of the bean, since it is severe on certain leading varieties of both canning and field beans and is increasingly becoming a limiting factor in bean culture in certain sections of the country.

(2) The general symptoms and effects of the disease are described, including symptoms of plants infected during the season and plants originating from infected seed.

(3) Well-marked symptoms develop at 20–28° C. with an incubation period of 8 to 15 days; a partial masking occurs at 28–32° C., together with an increased incubation period; total masking occurs at 12–18° C.

(4) Inoculation and other experiments indicate that there is only one mosaic disease of the bean *Phaseolus vulgaris* L., which appears to be limited to this one host plant.

(5) The virus appears to be present in all aerial vegetative parts of mosaic plants. In the seed it apparently is limited to the embryo.

(6) No transmission of the disease has been obtained through the soil or through contact of roots or of aerial parts of mosaic and healthy plants.

(7) A leaf-mutilation method of inoculation has been developed which has yielded from 80 to 100 per cent infection in the majority of cases.

Inoculation through cut roots has given infection in a low percentage of cases. Soaking healthy seed, whether dormant or germinating, in infective juice gave no infection.

(8) The virus has been successfully transmitted by certain aphids: *Aphis rumicis* L., *Myzus persicae* Sulz., *Macrosiphum solanifolii* Ashm., and by a species of mealybug. Fifteen or more infected aphids per plant gave a higher percentage of infection than did fewer, although a single viruliferous individual of either *A. rumicis* or *Myzus persicae* was found to cause infection in some cases.

(9) The bean leaf hopper, *Empoasca fabae* Le B., and other species of insects tested for bean-mosaic transmission have thus far given negative results.

(10) Great variations in the prevalence of bean mosaic were observed in a given locality. The occurrence and amount of the disease in a field are chiefly determined by (a) the number of mosaic plants originating from infected seed which may serve as primary sources of inoculum; (b) the relative prevalence of aphids which may act as virus vectors.

(11) The amount of field infection and the extent of the injury from mosaic can be greatly reduced by planting early enough to permit the plants to go through their period of vegetative activity and reach the blossoming stage before aphids become abundant. The amount of injury increases with the delay in date of planting. The late varieties, however, owing to their longer growing period, cannot get through the season without being subjected to insect transmission, thus a higher percentage of infection and greater injury result than with the early varieties.

(12) Bean mosaic is usually transmitted through the seed and so far as known, is carried over from one season to another only in infected seed. Thus the spread from one locality to another is largely through infected seed in commerce.

(13) A wide difference in the amount of seed transmission has been observed between early and late varieties, as high as 60 per cent infection having been found in the commercial seed of late susceptible varieties.

(14) Seeds from plants infected during the current season do not all show mosaic infection. In an individual pod some seeds may be infected and others not. The percentage of seed infection is correlated with the stage of the plant when infection occurs and decreases as the plant approaches the blossoming period. Plants infected after the blossoms are set show no seed infection.

(15) The viability of the seed does not appear to be affected by the presence of the virus.

(16) The date of planting has been found to affect the amount of seed transmission in a given variety. Early varieties planted early are more likely to escape seed infection than late varieties. Both varieties, planted at Madison between May 20 and 25, showed less seed infection than those planted on June 10 or later. In general, commercial seed of early varieties shows less seed infection than that of the late varieties.

(17) Plants of susceptible varieties are apparently susceptible to mosaic infection at any stage of their development after the early seedling stage. It was not found possible to obtain infection of Robust, a resistant variety. All other commercial varieties tested were found to be susceptible to infection and showed varying degrees of severity of leaf symptoms.

(18) The methods of control suggested are: (a) Production of mosaic-free seed, (b) testing of seed lots in advance and planting only seeds that are free or nearly free from infection, (c) the development and use of varieties reaching maturity early enough to escape seed infection and still produce good yields, (d) the development and use of resistant varieties.

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